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Indian Standard
SPECIFICATION FOR
GELATIN, MICROBIOLOGICAL GRADE

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SPECIFICATION FOR GELATIN, MICROBIOLOGICAL GRADE

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Indian Standard

SPECIFICATION FOR GELATIN, MICROBIOLOGICAL GRADE

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 16 January 1975, after the draft finalized by the Food Hygiene, Sampling and Analysis Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 Unless the ingredients used in media for microbiological work are of uniform quality, the results obtained would be erroneous and would be unreliable. Since the media used in different laboratories often differ greatly in their quality, the results of microbiological work at different laboratories cannot be compared. Therefore, with a view to unifying the practices of different laboratories dealing with microbiology and providing guidance to the indigenous manufacturers regarding the quality of various ingredients, it has been decided to bring out a series of Indian Standard specifications for ingredients commonly used in media for microbiological work.

0.3 Gelatin is obtained by partial hydrolysis of collagen derived from the skin, white connective tissues and bones of animals. Gelatin, foodgrade, is widely used as an emulsifying and thickening agent in various food products in the country. This is covered by IS : 5719-1970*. Gelatin conforming to lesser number of requirements but passing those mentioned in this standard can be used as an ingredient for microbiological media, for solidification of nutrient broth where temperature of incubation is not more than 20°C to 25°C and for the culture of organisms in dairy and milk products. It is also used as a diagnostic reagent for determining proteolysis by liquefaction of nutrient gelatin.

0.4 For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS : 2-1960†. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

*Specification for gelatin, food grade.

†Rules for rounding off numerical values (*revised*).

1. SCOPE

1.1 This standard prescribes requirements and methods of sampling and test for gelatin, microbiological grade.

2. REQUIREMENTS

2.1 Description — The material shall be in the form of sheets, flakes, shreds or coarse to fine powder. It shall be colourless, faintly yellow or amber in colour. It shall not contain any preservatives which will inhibit the growth of micro-organisms.

2.2 Solubility — The material shall be insoluble in cold water but shall swell and soften when immersed in it. It shall be soluble in hot water forming a jelly on cooling. It shall dissolve in acetic acid and in hot mixture of glycerol and water. It shall be insoluble in 95 percent alcohol, in chloroform and in ether solvent.

2.3 A hot solution of the material (1 in 40) shall be free from any disagreeable odour and shall be only slightly opalescent when observed in a layer of 2 cm thickness.

2.4 A dilute aqueous solution of the material shall pass the identification test given in Appendix A.

2.5 The material shall form a suitable gel with agar. The surface of the gel shall not show any physical distortion (scum-like surface). A gel obtained from 15 percent gelatin shall be firm and media containing this proportion of gelatin should withstand autoclaving at 115°C for 15 minutes without loss of gel strength.

2.6 The material shall pass the test for gelatin liquefaction prescribed in Appendix B. When tested on solid medium containing nutrient broth and gelatin, the gelatin should be liquefied by *Proteus vulgaris* at 22°C and shall be not liquefied by *salmonella*.

2.7 It shall also conform to the requirements given in Table 1.

3. PACKING, MARKING AND STORAGE

3.1 Packing — The material shall be securely packed in well-filled wide mouth containers with tightly fitting lids.

3.2 Marking — Each container shall be marked legibly to give the following information:

- a) Name of the material including the words 'Microbiological Grade',
- b) Name and address of the manufacturer,
- c) Minimum net content, and
- d) Batch or code number.

TABLE 1 REQUIREMENTS FOR GELATIN, MICROBIOLOGICAL GRADE

(Clause 2.7)

Sl No.	CHARACTERISTIC	REQUIREMENT	METHOD OF TEST, REF TO	
			Appendix of this Standard	CI No. of IS : 6854-1973*
(1)	(2)	(3)	(4)	(5)
i)	Moisture, percent by mass, <i>Max</i>	15	—	4
ii)	Total ash, percent by mass, <i>Max</i>	3	—	6
iii)	Nitrogen (on dry basis), percent by mass, <i>Min</i>	15	—	9
iv)	Arsenic (as As), mg/kg, <i>Max</i>	2	—	12
v)	Heavy metals, ppm, <i>Max</i>	50	C	—

*Methods of sampling and test for ingredients used in the media for microbiological work.

3.2.1 Each container may also be marked with the ISI Certification Mark.

NOTE — The use of the ISI Certification Mark is governed by the provisions of the Indian Standards Institution (Certification Marks) Act and the Rules and Regulations made thereunder. The ISI Mark on products covered by an Indian Standard conveys the assurance that they have been produced to comply with the requirements of that standard under a well-defined system of inspection, testing and quality control which is devised and supervised by ISI and operated by the producer. ISI marked products are also continuously checked by ISI for conformity to that standard as a further safeguard. Details of conditions under which a licence for the use of the ISI Certification Mark may be granted to manufacturers or processors, may be obtained from the Indian Standards Institution.

3.3 Storage — The material shall be stored in a cool and dry place.

4. SAMPLING

4.1 The representative samples of the material shall be drawn according to the method prescribed in 3 of IS : 6854-1973*.

5. TESTS

5.1 Tests shall be carried out by the methods prescribed in 2.4, 2.6 and in col 4 and 5 of Table 1.

*Methods of sampling and test for ingredients used in media for microbiological work.

5.2 Quality of Reagents — Unless specified otherwise, pure chemicals and distilled water (*see* IS : 1070-1960*) shall be employed in tests.

NOTE — ' Pure chemicals ' shall mean chemicals that do not contain impurities which affect the experimental results.

A P P E N D I X A

(*Clause 2.4*)

IDENTIFICATION TEST

A-1. PROCEDURE

A-1.1 To an aqueous solution (1 in 100) of gelatin, add trinitrophenol solution (1 g anhydrous material dissolved in 100 ml water) or a solution of potassium dichromate (1 in 15) previously mixed with one-fourth its volume of dilute hydrochloric acid. A pale yellow precipitate should be formed.

A-1.2 To an aqueous solution (1 in 5000) of gelatin, add freshly prepared tannic acid solution (1 g dissolved in 1 ml alcohol and diluted with water to 10 ml). Turbidity should be produced.

A P P E N D I X B

(*Clause 2.6*)

TEST FOR GELATIN LIQUEFACTION

B-1. STRAINS

B-1.1 *Proteus vulgaris* and *Salmonella typhi*

B-2. MEDIA AND METHOD

B-2.0 Any of the two media and methods may be used.

B-2.1 Nutrient Gelatin — The medium should contain meat extract (*see* IS : 6851-1973†) 0.3; peptone (*see* IS : 6853-1973‡) 0.5 g; gelatin, 12 g; and distilled water, 100 ml. First add gelatin to the water and allow to stand for 15 to 30 minutes and then heat gently to dissolve the gelatin. When the gelatin is completely dissolved, add meat extract and peptone, mix well to dissolve and adjust to pH 7.0. Distribute in approximately 6 ml amounts into clean test tubes of size approximately 12 cm × 2 cm and then sterilize by autoclaving at 115°C for 15 minutes. Special care is to be taken to ensure that the medium is not overheated.

*Specification for water, distilled quality (*revised*).

†Specification for meat extract, microbiological grade.

‡Specification for peptone, microbiological grade.

B-2.1.1 Using overnight growths of *P. vulgaris* and *S. typhi* on nutrient agar (see B-2.2), inoculate, as stab cultures with a straight wire individual tubes of nutrient medium, separately with the bacterial strains. Incubate the tube containing *P. vulgaris* at 22°C and the tube containing *S. typhi* at 37°C. Observe for liquefaction of the gelatin daily up to 7 days the cultures of *P. vulgaris* and up to 14 days the cultures of *S. typhi*. Uninoculated tubes of nutrient gelatin incubated parallelly with each set of uninoculated medium serves as control. Observe the presence or absence of liquefaction of gelatin by keeping the incubated tubes in an ice-bath or refrigerator for 2 to 3 hours to allow the gelatin medium in the uninoculated tubes to become firm.

B-2.2 Gelatin Agar—The medium contains gelatin 0.4 g; distilled water, 5 ml; and sterilized nutrient agar, 100 ml. The nutrient agar is prepared by dissolving with heat in 100 ml of distilled water, meat extract (see IS:6851-1973*) 1.0 g; peptone (see IS:6853-1973†) 1.0 g; and Sodium Chloride (NaCl) 0.5 g. The pH should be adjusted to 8.0 to 8.4, then boiled for 10 minutes and filtered. The pH now adjusted to 7.2 to 7.4 and the medium sterilized by autoclaving at 115°C for 15 minutes. Soak the gelatin in the distilled water and when thoroughly softened, add to melted sterilized nutrient agar. Mix well and sterilize by autoclaving at 115°C for 15 minutes. Distribute into sterilized glass petridishes of size approximately 10 cm diameter.

B-2.2.1 Using overnight growths of *P. vulgaris* and *S. typhi* on nutrient agar, inoculate individual petridish of gelatin agar medium separately with the bacterial strains. Incubate the petridish containing *P. vulgaris* at 22°C and that containing *S. typhi* at 37°C, both for 7 days. To observe liquefaction of gelatin, flood the surface with 5 to 10 ml acid mercuric chloride solution (prepared by mixing 12.0 g of mercuric chloride in 80.0 ml distilled water, then adding 16.0 ml of concentrated HCl and shaking well until solution is complete) when clear zones around the colonies of bacteria indicate areas of gelatin hydrolysis.

APPENDIX C

[Table 1, Item (v)]

DETERMINATION OF HEAVY METALS

C-1. REAGENTS

C-1.1 Ammonia Solution 9.5 to 10.5 Percent—Dilute 400 ml concentrated ammonia with water to make 1000 ml.

*Specification of meat extract, microbiological grade.

†Specification of peptone, microbiological grade.

C-1.2 Concentrated Hydrochloric Acid — See IS : 265-1962*.

C-1.3 Hydrochloric Acid — 1 N.

C-1.4 Concentrated Nitric Acid — See IS : 264-1968†.

C-1.5 Acetic Acid (1 N) — Dilute 60 ml of glacial acetic acid with water to make 1 000 ml.

C-1.6 Hydrogen Sulphide, Saturated Solution — Pass hydrogen sulphide gas into cold water. Store it in small, dark amber-coloured bottles filled nearly to the top. It should possess a strong odour of hydrogen sulphide and should produce at once a copious precipitate of sulphur when added to an equal volume of 9 percent aqueous solution of ferric chloride. Store in a cold, dark place.

C-1.7 Lead Nitrate Stock Solution — Dissolve 159.8 mg of lead nitrate in 100 ml of water to which has been added 1 ml of nitric acid, dilute it with water to 1 000 ml. Prepare and store this solution in glass containers free from soluble lead salts.

C-1.8 Standard Lead Nitrate Solution — Dilute 10 ml of lead nitrate stock solution, accurately measured, with water to 100 ml. This solution contains the equivalent of 0.01 mg of lead.

C-1.9 Solution A — Into one of two 50 ml Nessler cylinders, pipette 25 ml of standard lead nitrate solution. Adjust the pH with ammonia solution between 3 and 4 using short range pH indicator paper. Dilute with water to 40 ml and mix.

C-1.10 Solution B — In the second cylinder place 25 ml of the solution prepared for the test. Adjust the pH with diluted acetic acid or ammonia solution between 3 and 4 using short-range pH indicator paper. Dilute with water to 40 ml and mix.

C-2. PROCEDURE

C-2.1 To the ash obtained from igniting 5 g of material, add 2 ml of hydrochloric acid and 0.5 ml of nitric acid. Evaporate on a steam bath to dryness. To the residue, add 1 ml of 1 N hydrochloric acid and 15 ml of water and warm for a few minutes. Filter and wash with water to make the filtrate to 50 ml.

C-2.2 Add 10 ml of freshly prepared saturated hydrogen sulphide solution to each of the 2 cylinders containing Solution A and Solution B, mix (preferably with a stirring rod having a loop at the lower end), allow to stand for 5 minutes, and view downwards over a white surface: the colour of Solution B should be no darker than that of Solution A.

*Specification for hydrochloric acid (*revised*).

†Specification for nitric acid (*first revision*).